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Phlebopus spongiosus sp. nov. (*Boletales, Boletinellaceae*) with a sponge-like tissue

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Abstract – A new species, *Phlebopus spongiosus*, is described with a peculiar sponge-like tissue in which the hollow spaces in the tubes are filled in by pleurocystidia-like elements, and a thin membrane of dissepiments encloses the immature pores. It occurs in citrus orchards (*Citrus maxima*) in southern Vietnam.

Key words – tropics, black bolete, edible mushroom, crypta

Introduction

Heim (1936) proposed *Phlebopus* as a subgenus of *Boletus* to accommodate *Boletus colossus* R. Heim. Singer (1936) elevated *Phlebopus* to genus with *P. colossus* (R. Heim) Singer as type species. Subsequently, Heinemann & Rammeloo (1982), Singer et al. (1983), and Singer (1986) emended the generic limits to encompass all taxa of *Phaeogyroporus* (Singer 1944). With 12 species (Kirk et al. 2008: 522), *Phlebopus* was classified in the *Boletinellaceae* (*Boletales*) by Binder & Bresinsky (2002).

Phlebopus is a tropical or subtropical genus with boletoid basidiomata producing an olivaceous brown spore print, ellipsoidal smooth basidiospores

(light microscope), and hyphae with clamp connections. In Southeast Asia, the two dominant edible taxa — *P. portentosus* (Berk. & Broome) Boedijn (Saccardo 1888, Boedijn 1951) and *P. marginatus* Watling & N.M. Greg. (Saccardo 1888, Watling & Gregory 1988) — are regarded as conspecific by Watling & Gregory (1988) and Watling & Li (1999).

During surveys of mycobiota in southern Vietnam, we collected in several orchards a taxon with a habit reminiscent of *P. portentosus*. In the present paper, this taxon is described and illustrated as new to science.

Materials & methods

Geographical coordinates of localities were determined by GPS (Garmin – eTrex Legend®) and Google Earth 5.0.11733.9347 software. Color notation in parentheses was determined using the Methuen Handbook of Colour (Kornerup & Wanscher 1981).

The microscopic characteristics were observed using a Nikon Labophot-2 equipped with DIC (Differential Interference Contrast) optics after hand-sectioning and mounting with 10% ammonium hydroxide aqueous solution. Abbreviations used: Q = mean length/width ratio measured from "n" number of spores; m = mean spore length and width; *P.* = *Phlebopus*.

A strain isolated from basidioma hyphae is maintained on modified Melin and Norkrans medium (Brundrett et al. 1996: 224), 20°C, in darkness.

All specimens are deposited in the herbarium of the Natural History Museum and Institute, Chiba, Japan (CBM).

Taxonomy

Phlebopus spongiosus Pham & Har. Takah., **sp. nov.** Pl. 1–6

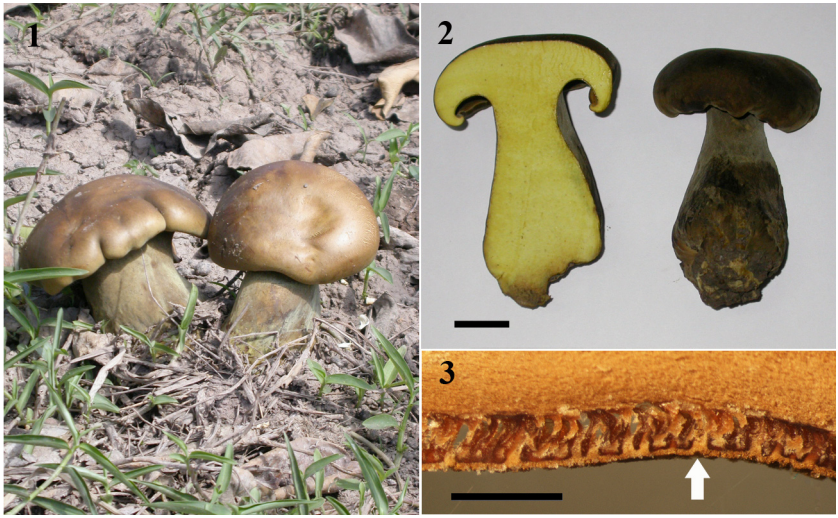
Mycobank MB 518549

Differs from other *Phlebopus* species by sponge-like tissues with cystidioid elements filling hollow spaces in immature tubes, a thin membrane of subcylindrical dissepiments enclosing the immature pores, and a dark brown spore print.

Type: Vietnam. TienGiang: MyTho City, Highway 1A (UTM, 0646165, 1151001), 20 Jan 2006, N.D.H Pham (**Holotype**, CBM FB-38014)

Etymology: From the Latin, referring to the sponge-like tissue

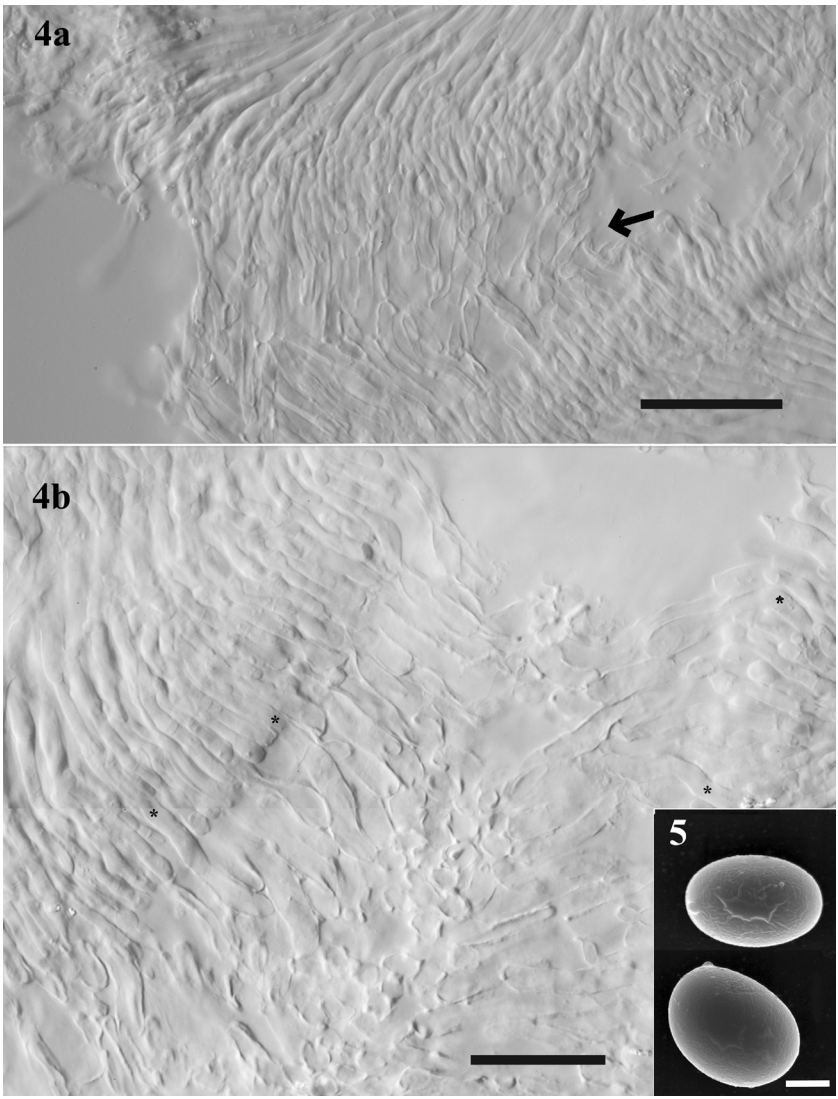
Macrocharacters – Pileus 50–120(–500) mm broad, at first hemispherical with incurved margin, then becoming plano-convex to convex with decurved margin; surface dry, at first subtomentose, then glabrescent; brown to yellowish brown (5D7-8 to 5E7-8) overall, sometimes tinged with olive (3F4-5) on old specimens. Flesh 10–20 mm thick at the center of pileus, soft, yellow (3A7 to 3B7-8) to vivid yellow (3A8), in places gradually bluing when exposed; odor indistinct when fresh; taste after cooking slightly like persimmon rind. Hymenophore decurrent to subdecurrent at first, then more or less depressed around the stipe; tubes ≤ 3 mm long in dried material, vertically arranged; hymenium usually with two thirds of the tubes filled in with yellowish orange



Figures 1–3. *Phlebopus spongiosus*. 1: Basidiomata in its habitat. 2: Vertical section of the immature basidioma; bar = 2 cm. 3: Vertical section of the hymenophore in dried specimens showing the sponge-like tissue arrow); bar = 1 mm.

(5A7-8 to 5B7-8) to yellowish brown (5E5-6 to 5F5-6), sponge-like tissue projecting from the sides of the tube walls, but this occluding tissue disappearing in old specimens; pores small (1–3 per mm), angular to subcircular, more elongated around the stipe, covered by a thin membrane in young specimens, brown (7D8-7E8) when young, then brown (7E5-6) to dark brown (7F5-6). Stipe 80–150 × 15–50 mm, central, subequal or somewhat enlarged at the base, sometimes tapering downward, solid; surface dry, subtomentose to tomentose, longitudinally striate toward the base; entirely yellowish brown (5E4-8 to 5F4-8), becoming darker where handled; basal mycelium brownish yellow. Spore print dark brown to reddish brown (8F5-6).

Microcharacters – Basidiospores m = 8.3 × 6.4 µm [7.9–8.8(–9.1) × 5.9–7.1(–7.4) µm, n = 47, Q = 1.30], ovoid to shortly ellipsoid, smooth under the light microscope, brown (5D6-7), inamyloid, thick-walled. Basidia 21–27 × 8–10 µm (without sterigmata), clavate, 4-spored; sterigmata 3–4 µm long. Hymenophoral trama divergent-bilateral of the *Boletus* subtype, hyaline, with gelatinized hyphae 4.5–10 µm diam.; mediostratum 80–110 µm diam. when young, 40–60 µm diam. at maturity. Sponge-like tissue composed of heteromorphous terminal cells occasionally protruding into the tube lacunae from the sides of the tube walls; constituent elements numerous, (29–)45–53 × 4.5–7.4 µm, cystidioid but not truly pleurocystidia, broadly clavate or fusoid-ventricose to ventricose-rostrate, thin-walled, with yellow (4B-5 to 4A-6)



Figures 4–5. *Phlebopus spongiosus*. 4: Vertical section through part of the hymenophore showing the sponge-like tissue (arrow) made up of heteromorphous terminal cells (*) unusually protruding into the lacunae of the tubes from the sides of the tube walls; bar = 50 µm in a, = 30 µm in b. 5: SEM figures of basidiospores; bar = 2.5 µm.

intracellular pigment, converging toward the center of the tube chamber at least in young specimens but gradually disrupted. Dissepiments resembling

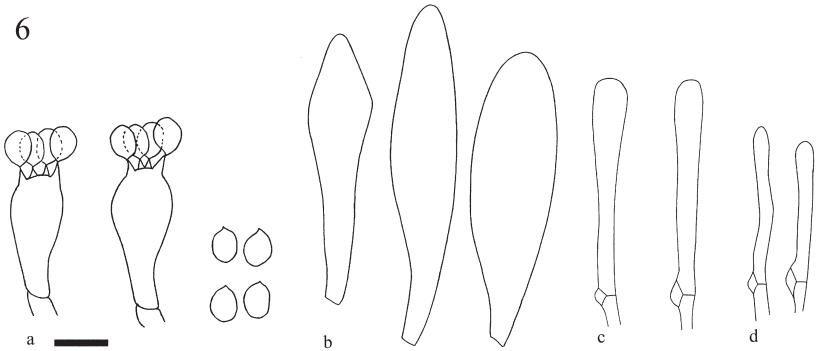


Figure 6. *Phlebopus spongiosus*. A: Basidiospores and basidia. B: Heteromorphous terminal cells protruding into the lacunae of the tubes from the sides of the tube walls. C: Dissepiments. D: Terminal cells of the pileipellis. Bar = 10 μm.

cheilocystidia, remarkably projecting beyond the hymenium, 25–33(–42) × 4–5(–6.5) μm, subcylindrical to cylindrical-clavate, thin-walled, distinctly clamped at base, with yellow (4B-5 to 4A-6) intracellular pigment, forming the membranous thin layer on the immature pore surface but gradually collapsing. Pileipellis consisting of repent, appressed, interwoven hyphal elements; terminal cells 22–29 × 3.3–4.7 μm, cylindrical, thin-walled, with yellow (4B7-8) intracellular pigment, golden yellow to brownish (5B7 or 5C7) in the wall mass. Pileitrama composed of cylindrical, loosely interwoven hyphae 4-6 μm diam., colorless or greyish yellow, smooth, inamyloid, thin-walled. Stipitipellis densely constructed, brown (6D8 or 6E7); terminal cells similar to those of the pileipellis. Stipe trama composed of longitudinally arranged, cylindrical hyphae 2-4.5 μm diam., thin-walled, colorless or brownish (6C4-6C5), inamyloid, rather compactly arranged compared to the pileitrama. Clamp connections constantly present in all tissues and in vitro.

Habitat – Almost year-round, solitary to scattered in citrus farms, (dominated by *Citrus maxima* (Burm.) Merr).

Additional specimens examined: **VIETNAM**. **TienGiang**: MyTho City, Highway 1A (UTM, 0646165, 1151001), 25 Jun 2007, N.D.H Pham (CBM FB-38670; culture Phle01, Laboratory of Microbiology, Biotechnology Center of HoChiMinh City). **HoChiMinh City**: TanPhu Dist., Cautre Company, 16 May 2007, N.D.H Pham (CBM FB-38017); Dist. 12, Biotechnology Center of HoChiMinh City (10°50′58.95″N, 106°37′03.89″E), 30 Mar 2008, N.D.H Pham (CBM FB-38671).

Discussion

The most distinctive features of *Phlebopus spongiosus* are the sponge-like tissue composed of numerous fusoid-ventricose to ventricose-rostrate pleurocystidioid elements, the subcylindrical to subclavate dissepiments

forming a thin membrane that covers the immature pores, and the dark-brown spore print not found in any other known *Phlebopus* species.

Although its dark brown spore print appears foreign to *Phlebopus* sensu Singer (1986), the shortly ellipsoid smooth basidiospores and clamp connections merit placing *P. spongiosus* within *Phlebopus* (Singer 1986).

Its medium to large dark yellowish-brown boletoid basidioma bears a superficial resemblance to the Southeast Asian *P. portentosus* (Boedijn 1951, Heinemann & Rammeloo 1982, Singer et al. 1983) and *P. marginatus* originally described from Australia (Watling & Gregory 1988, Grgurinovic 1997). Both taxa differ from *P. spongiosus* in their olivaceous brown spore print and lack of distinct hymenial cystidia (Boedijn 1951; Pegler 1986; Watling & Li 1999).

It should be noted that *P. spongiosus* basidiomata are sometimes accompanied by a crust-like mycelial structure around nearby citrus tree roots reminiscent of "crypta" as defined by Singer ("sleeve-like formations around tree roots in tropical and subtropical conditions;" 1986: 10). Further critical investigations, including cultural and molecular studies, are needed to confirm its relationship with *P. spongiosus* basidiomata.

The crypta-like mycelium of *P. spongiosus* is comparable to that of three neotropical taxa: *P. tropicus* (Rick) Heinem. & Rammeloo (Singer 1944, Singer & Digilio 1957, Heinemann & Rammeloo 1982, Singer et al. 1983), *P. beniensis* (Singer & Digilio) Heinem. & Rammeloo (Heinemann & Rammeloo 1982, Singer et al. 1983), and *P. brasiliensis* Singer (Singer et al. 1983). Those taxa differ from *P. spongiosus* mainly by lacking distinct hymenial cystidia and having olivaceous-brown spore print. Moreover, according to the *Phlebopus* key by Singer et al. (1983), the wide pores (≥1 mm diam.) or boletinoid/subgyrose hymenophore separate *P. tropicus* from *P. spongiosus*. Miller et al. (2000) noted that Puerto Rican *P. beniensis* specimens had well-developed cheilocystidia but provided no information regarding the presence or absence of pleurocystidia.

Phlebopus spongiosus also shares well developed fusoid-ventricose to ventricose-rostrate pleurocystidia-like elements with *P. cystidiosus* Heinem & Rammeloo from Ethiopia (Heinemann & Rammeloo 1982). However, the lack of clamp connections causes *P. cystidiosus* to seem out of place in *Phlebopus.* Its glabrous non-reticulate stipe, ellipsoid basidiospores, and absence of clamp connections make *P. cystidiosus* seem more closely related to *Rubinoboletus* Pilát & Dermek (Heinemann & Rammeloo 1983, Watling & Gregory 1988, Li & Watling 1999, Watling & Li 1999).

The thin membrane enclosing the immature pores might also suggest a relationship between *P. spongiosus* and the secotioid boletes (Thiers 1984). However, the secotioid boletes are generally associated with an often misshapen pileus, irregularly oriented tubes, and a typically less-developed stipe, characters not seen in *P. spongiosus*.

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